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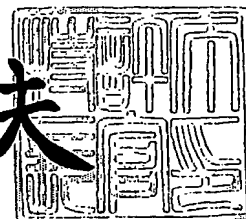
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【物件名】 要約書 1

【プルーフの要否】 要

【書類名】 明細書

【発明の名称】 4 s 期神経芽細胞腫から単離された核酸

【特許請求の範囲】

【請求項 1】 配列表の配列番号 1 ないし 1 7 4 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸。

【請求項 2】 配列表の配列番号 1 ないし 1 4 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる、請求項 1 に記載の核酸。

【請求項 3】 請求項 1 または 2 に記載の核酸に相補的な核酸。

【請求項 4】 請求項 1 ないし 3 のいずれか 1 項に記載の核酸と、ストリンジェントな条件下でハイブリダイズする核酸。

【請求項 5】 以下の(a)或いは(b)の核酸を含む核酸プローブ：

- (a) 配列表の配列番号 1 ないし 1 7 4 に記載の核酸配列からなる群より選ばれる 1 つの配列の全長若しくは一部からなる核酸、またはそれに相補的な核酸；
- (b) 配列表の配列番号 1 ないし 1 7 4 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、またはそれに相補的な核酸。

【請求項 6】 以下の(a)或いは(b)の核酸を含む請求項 5 に記載の核酸プローブ：

- (a) 配列表の配列番号 1 ないし 1 4 に記載の核酸配列からなる群より選ばれる 1 つの配列の全長若しくは一部からなる核酸、若しくはそれに相補的な核酸；
- (b) 配列表の配列番号 1 ないし 1 4 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、若しくはそれに相補的な核酸。

【請求項 7】 請求項 5 または 6 に記載の核酸プローブを有効成分として含有する 4 s 期神経芽細胞腫の診断剤。

【請求項 8】 以下の(a)或いは(b)の DNA を含むプライマー：

- (a) 配列表の配列番号 1 7 5 ないし 1 0 7 6 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる DNA、またはそれに相補的な DNA；
- (b) 配列表の配列番号 1 7 5 ないし 1 0 7 6 に記載の核酸配列からなる群より選

ばれる 1 つの配列からなる DNA とストリンジェントな条件下でハイブリダイズする DNA、またはそれに相補的な DNA。

【請求項 9】 以下の (a) 或いは (b) の DNA を含むプライマー：

(a) 配列表の配列番号 175 ないし 202 に記載の核酸配列、および配列番号 519 ないし 540 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる DNA、若しくはそれに相補的な DNA、または配列表の配列番号 785 ないし 798 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる DNA、若しくはそれに相補的な DNA；

(b) 配列表の配列番号 175 ないし 202 に記載の核酸配列、および配列番号 519 ないし 540 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる DNA と、または配列表の配列番号 785 ないし 798 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる DNA とストリンジェントな条件下でハイブリダイズする DNA、若しくはそれに相補的な DNA。

【請求項 10】 請求項 8 または 9 に記載のプライマーを一組、有効成分として含有する 4 s 期神経芽細胞腫の診断キット。

【請求項 11】 神経芽細胞腫の臨床組織サンプルから配列表の配列番号 1 ないし 14 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸の有無を検出することを特徴とする、4 s 期神経芽細胞腫の判定方法。

【請求項 12】 固相支持体に、配列番号 1 ないし 174 に記載の核酸配列の全長若しくは一部からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイ。

【請求項 13】 固相支持体に、配列番号 175 ないし 202 に記載の核酸配列、配列番号 519 ないし 540 に記載の核酸配列、および配列番号 785 ないし 798 に記載の核酸配列からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイ。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】

本発明は、ヒト神経芽細胞腫において発現する遺伝子に由来する核酸類に関す

る。さらに詳しくは、本発明は、4 s 期のヒト神経芽細胞腫において発現する遺伝子に由来する核酸類に関する。さらに、本発明は、このような核酸およびそれらの断片、あるいはそれらの組み合わせを利用した核酸プローブ、プライマーまたは核酸マイクロアレイ等からなる、4 s 期神経芽細胞腫の診断剤および診断キット、さらには上記遺伝子からの核酸配列情報に基づく癌細胞のプログラム細胞死機構の解明に関する。

【0002】

【従来の技術】

（腫瘍形成と遺伝子）

個々の腫瘍にはそれぞれの個性があり、発癌の基本的な原理は同じであっても、その生物学的特性は必ずしも同じではない。近年、癌の分子生物学や分子遺伝学が急速に進歩し、発癌やいわゆる腫瘍細胞のバイオロジーが遺伝子レベルで説明できるようになってきた。

【0003】

（神経芽細胞腫）

神経芽細胞腫は末梢交感神経系細胞に由来する交感神経節細胞と副腎髄質細胞から発生する小児癌である。この交感神経系細胞は発生初期の神経堤細胞が腹側へ遊走し、いわゆる交感神経節が形成される場所で分化成熟したものである。その一部の細胞はさらに副腎部へ遊走し、先に形成されつつある副腎皮質を貫通して髄質部に達し、そこで髄質を形成する。神経堤細胞は、ほかの末梢神経細胞の起源ともなっており、後根神経節（知覚神経）、皮膚の色素細胞、甲状腺C細胞、肺細胞の一部、腸管神経節細胞などへ分化する。

【0004】

（神経芽細胞腫の予後）

神経芽細胞腫は多彩な臨床像を示すことが特徴である（非特許文献1参照）。例えば、1歳未満で発症する神経芽細胞腫は非常に予後が良く、大部分が分化や細胞死を起こして自然退縮する（予後良好型ともいう）。現在、広く実施されている生後6か月時の尿のマススクリーニングで陽性となる神経芽細胞腫の多くは、この自然退縮を起こしやすいものに属する。一方、1歳以上で発症する神経芽

細胞腫は悪性度が高く、多くの場合、治療に抵抗して患児を死に至らしめる（予後不良型ともいう）。1歳以上の悪性度の高い神経芽細胞腫は、体細胞突然変異（Somatic mutation）が起こり、モノクローナルであるのに対し、自然退縮する神経芽細胞腫では生殖細胞突然変異（germ line mutation）のみの遺伝子変異でとどまっているとの仮説もある（非特許文献2参照）。さらに、臨床的にこれらの型の間位置する中間型の神経芽細胞腫もある。

【0005】

腫瘍の進行度からこれら神経芽細胞腫を分類すると以下ようになる。

- 1期：副腎または交感神経節に原発し、限局している。
- 2期：原発巣に限局した腫瘍と局部リンパ節転移のみを有する。リンパ節転移は正中線を越えない。
- 3期：腫瘍が正中線を越えて対側に浸潤またはリンパ節転移をきたす。
- 4期：骨、骨髄、眼窩部に遠隔転移を起こす。
- 4s期：1歳未満に発症し、骨髄、皮膚、肝に遠隔転移する。

【0006】

予後良好型の神経芽細胞腫は、1、2、4s期の腫瘍であり、予後不良型および中間型の神経芽細胞腫は、3、4期の腫瘍である。4s期の腫瘍は、特異的であり、通常生後数ヶ月の乳児に発症し、急速に腫瘍が増殖転移するが、突然増殖が止まり、その後は自然に腫瘍が消失する。このように、自然退縮する腫瘍と悪性増殖する腫瘍との間の違いは、発症年齢と転移部位、さらに進行度が明らかに異なる。

【0007】

（神経芽細胞腫の予後を推定する遺伝子）

最近の分子生物学的研究の進展により、神経成長因子（nerve growth factor：NGF）の高親和性レセプターであるTrkAの発現が分化と細胞死の制御に深くかかわっていることが明らかとなってきた（非特許文献3参照）。Trkは神経栄養因子の高親和性受容体で、膜貫通型受容体であり、Trk-A、B、Cの3つが主なものである。

【0008】

Trkファミリー受容体は、中枢神経および末梢神経系において、特異的な神経細胞の分化と生存維持に重要な役割を果たしている（非特許文献4参照）。腫瘍細胞の生存や分化はTrkチロシンキナーゼやRetチロシンキナーゼからのシグナルで制御されている。なかでも、TrkA受容体の役割は最も重要で、予後良好型の神経芽細胞腫ではTrkAの発現が著しく高く、これからのシグナルが腫瘍細胞の生存・分化、または細胞死（アポトーシス）を強く制御している。一方、予後不良型の神経芽細胞腫では、TrkAの発現が著しく抑えられており、これに代わってTrkBあるいはRetからのシグナルが生存の促進という形で腫瘍の進展を助長している。

【0009】

また、神経の癌遺伝子であるN-mycの増幅が神経芽細胞腫の予後に関連していることも明らかになってきた（非特許文献5参照）。この遺伝子は神経芽細胞腫で初めてクローニングされたが、正常細胞や予後良好型の神経芽細胞腫では通常1倍体当たり1つしか存在しないのに対し、予後不良型の神経芽細胞腫においては数十倍に増幅されているのが見つかった。

【0010】

上記の遺伝子以外にも、予後良好型の神経芽細胞腫で高発現する遺伝子として、CD44、PTN、caspase等が知られており、また予後不良型の神経芽細胞腫で高発現する遺伝子としては、SVV (survivin)、MK (midkine) 等が知られている。

【0011】

さらに、本発明者らは、予後良好型の神経芽細胞腫において、一群の新規な遺伝子が高発現していることを見出し（特許文献1参照）、また対照的に予後不良型の神経芽細胞腫において、別の一群の新規な遺伝子が高発現していることを見出した（特許文献2参照）。

【0012】

【特許文献1】

国際公開PCT/JP01631号パンフレット

【特許文献2】

国際公開 PCT/JPO1629 号パンフレット

【0013】

【非特許文献 1】

中川原, 「神経芽腫の発生とその分子機構」, 小児内科, 1998 年, 第 30 巻, p. 143

【非特許文献 2】

ヌーソン・エー・ジーら (Knudson AG et al.), 「4 s 期神経芽細胞腫の退縮—遺伝学的仮説 (Regression of neuroblastoma IV-S: A genetic hypothesis)」, ニューイングランド・ジャーナル・オブ・メディスン (N. Engl. J. Med.), 米国, 1980 年, 第 302 巻, p. 1254

【非特許文献 3】

ナカガワラ・エー (Nakagawara A.), 「NGF そして神経芽細胞腫 (The NGF story and neuroblastoma)」, メディカル・ペディアトリック・オンコロジー (Med. Pediatr. Oncol.), 米国, 1998 年, 第 31 巻, p. 113

【非特許文献 4】

中川原等, 「神経芽細胞腫におけるニューロトロフィン受容体の発現と予後」, 小児外科, 1997 年, 第 29 巻, p. 425-432

【非特許文献 5】

中川原, 「脳・神経腫瘍の多段階発癌」, モレキュラー・メディスン (Molecular Medicine), 1999 年, 第 364 巻, p. 366

【0014】

【発明が解決しようとする課題】

しかしながら、現在までに 4 s 期神経芽細胞腫において発現する（特に、特異的に）遺伝子についてはほとんど知られていなかった。さらに、上記のように 4 s 期神経芽細胞腫は自然退縮するので、この原因となる遺伝子の同定も急務である。

【0015】

本発明は、上記従来技術の有する課題に鑑みてなされたものであり、一般的に

神経芽細胞腫の予後良不良に関係する遺伝子の核酸配列を明らかにし、そのような遺伝子情報の提供および予後良不良に関する診断を可能とすることを目的とする。本発明は、特定のには神経芽細胞腫の予後を診断し、該細胞腫の進行度分類を行い、4 s 期神経芽細胞腫の判定を可能とすることを目的とする。

【0016】

【課題を解決するための手段】

本発明者らは鋭意研究した結果、ヒト神経芽細胞腫の予後を検定し、予後良好型および予後不良型の臨床組織の各々から cDNA ライブラリーを作製することに成功した。これら2種類の cDNA ライブラリーから各々約2400個のクローンをクローニングし、神経芽細胞腫の予後の良悪によって分類し、それぞれのサブセットで遺伝子のプロファイリングを行った。

【0017】

そこで本発明者らは、前記サブセット間で示差的に発現し、かつ予後良好型の臨床組織でのみ発現が増強している遺伝子群を見いだした。加えて、本発明者は、予後不良型の臨床組織でのみ発現が増強している遺伝子群をも見いだした。かかる知見に基づき、本発明者は少なくとも予後良好型の臨床組織または、予後不良型の臨床組織でのみ発現が増強している遺伝子を検出およびクローニングするための核酸配列情報を提供することを可能とした。

【0018】

さらに、本発明者らは、4 s 期神経芽細胞腫の臨床組織から同様に cDNA ライブラリーを作製することに成功した。このライブラリーから約2700個のクローンをクローニングした。このライブラリーのサブセットと、予後良好型および予後不良型の臨床組織からのライブラリーのサブセットを解析して、これらのサブセット間で発現する約1600個の遺伝子のプロファイリングを行った。その結果、前記サブセット間で示差的に発現する452個の遺伝子を同定した。これらの遺伝子をシーケンスしたところ、308個の新規な遺伝子と、残り144個の既知の遺伝子とから成っていた。前記遺伝子をそれぞれのサブセット間での発現パターンに従って、分類し7つの群にグループ化した。

【0019】

かかる知見に基づき、本発明者らは、4 s 期神経芽細胞腫を特徴づける発現パターンを呈する遺伝子を検出およびクローニングするための遺伝子情報（核酸配列情報等）を提供することを可能とした。さらに該核酸配列情報に基づき、神経芽細胞腫の予後診断法（特に、進行度分類）を、4 s 期神経芽細胞腫の判定を含めて、可能とする診断剤や診断キットを提供することを可能とし、本発明を完成した。

【0020】

すなわち、本発明によれば、配列表の配列番号 1 ないし 174 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸が提供される。

【0021】

好ましい核酸は、前記配列番号 1 ないし 174 のうち、配列番号 1 ないし 14 のいずれか一つに記載の核酸配列からなる核酸である。

【0022】

また、本発明によれば、上記これらの核酸に相補的な核酸も提供される。

【0023】

また、本発明によれば、上記の核酸と、またはそれに相補的な核酸とストリンジェントな条件下でハイブリダイズする核酸が提供される。

また、本発明によれば、

以下の(a)或いは(b)の核酸を含む核酸プローブが提供される：

(a)配列表の配列番号 1 ないし 174 に記載の核酸配列からなる群より選ばれる 1 つの配列の全長若しくは一部からなる核酸、またはそれに相補的な核酸；

(b)配列表の配列番号 1 ないし 174 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、またはそれに相補的な核酸。

【0024】

好ましくは、前記(a)或いは(b)の核酸がDNAである。

【0025】

また、好ましくは、前記(a)または(b)の核酸が配列番号 1 ないし 14 に記載の

核酸配列からなる群より選ばれる 1 つの配列からなる核酸である。

【0026】

また、本発明によれば上記の核酸プローブを有効成分として含有する 4 s 期神経芽細胞腫の診断剤が提供される。

【0027】

さらに、本発明によれば、

以下の(a)或いは(b)のDNAを含むプライマーが提供される：

(a)配列表の配列番号175ないし1076に記載の核酸配列からなる群より選ばれる1つの配列からなるDNA、またはそれに相補的なDNA；

(b)配列表の配列番号175ないし1076に記載の核酸配列からなる群より選ばれる1つの配列からなるDNAとストリンジェントな条件下でハイブリダイズするDNA、またはそれに相補的なDNA。

【0028】

好ましくは、前記(a)或いは(b)のDNAが配列番号175ないし202に記載の核酸配列、および配列番号519ないし540に記載の核酸配列からなる群より選ばれる1つの配列からなるDNA、または配列表の配列番号785ないし798に記載の核酸配列からなる群より選ばれる1つの配列からなるDNAである。

【0029】

また、本発明によれば上記のプライマーを一組、有効成分として含有する 4 s 期神経芽細胞腫の診断キットが提供される。

【0030】

また、本発明によれば神経芽細胞腫の臨床組織サンプルから配列表の配列番号1ないし14に記載の核酸配列からなる群より選ばれる1つの配列からなる核酸の有無を検出することを特徴とする、4 s 期神経芽細胞腫の判定方法が提供される。

【0031】

加えて、本発明によれば固相支持体に、配列表の配列番号1ないし174に記載の核酸配列からなる核酸の全長若しくは一部からなる核酸を複数個組み合わせ

て、固定してなる核酸マイクロアレイが提供される。

【0032】

また、本発明によれば固相支持体に、配列番号175ないし202に記載の核酸配列、配列番号519ないし540に記載の核酸配列、および配列番号785ないし798に記載の核酸配列からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイが提供される。ここで、記載された配列番号を有する核酸配列からなる核酸の複数個の任意の組み合わせが用いられる。

【0033】

【発明の実施の形態】

以下、本発明に係る神経芽細胞腫に発現する遺伝子（以下、「本発明の遺伝子」という）に由来する核酸（以下、「本発明の核酸」という）について、その用途を含めて、本発明の好適な実施の形態を参照して、詳細に説明する。

【0034】

本発明の核酸は、上述のごとく本発明の遺伝子に由来するものであり、該遺伝子を構成するか或いは該遺伝子からインビボまたはインビトロの過程によって得られる。該核酸の鎖長には特に制限はなく、本明細書では前記遺伝子の一部に対応する核酸断片を含めて「本発明の核酸」という。核酸の鎖長が短い場合、その核酸は化学的手法で合成することができる。

【0035】

本明細書で使用する「核酸」という用語は、例えばDNAまたはRNA、或いはそれらから誘導された活性なDNA若しくはRNAでありうるポリヌクレオチドを指し、好ましくは、DNAまたはRNAを意味する。特に好ましい核酸は、本明細書中に開示されるヒトcDNA配列と同一か、またはそれに相補的な配列を有する。

【0036】

また、本発明で使用する「ストリンジェントな条件下でハイブリダイズする」という用語は、2つの核酸（または断片）が、サムブルックら（Sambrook, J.）の「大腸菌におけるクローン遺伝子の発現（Expression of cloned genes in E. coli）」、モレキュラー・クローニング：ア・ラボラトリー・マニュアル（Mol

ecular Cloning: A laboratory manual), 米国, コールド・スプリング・ハーバー・ラボラトリー・プレス (Cold Spring Harbor Laboratory Press), 1989年, p. 9. 47-9. 62, p. 11. 45-11. 61に記載されたハイブリダイゼーション条件下で、相互にハイブリダイズすることを意味する。

【0037】

より具体的には、前記「ストリンジェントな条件」とは、約45℃において6.0×SSCでハイブリダイゼーションを行った後に、50℃で2.0×SSCで洗浄することを指す。ストリンジェンシーの選択のため、洗浄工程における塩濃度を、例えば低ストリンジェンシーとしての約2.0×SSC、50℃から、高ストリンジェンシーとしての約0.2×SSC、50℃まで選択することができる。さらに、洗浄工程の温度を低ストリンジェンシー条件の室温、約22℃から、高ストリンジェンシー条件の約65℃まで高くすることができる。

【0038】

また、本明細書で使用する「核酸」という用語は、単離された核酸を指し、これは組換えDNA技術により調製された場合は細胞物質、培養培地を実質的に含有せず、化学合成された場合には前駆体化学物質またはその他の化学物質を実質的に含まない、核酸またはポリペプチドを指す。

【0039】

本明細書で使用する「予後良好型」とは、ヒト神経芽細胞腫のうち、腫瘍が局限して存在するか、または退縮や良性の交感神経節細胞腫になった状態を指し、N-myc その他腫瘍マーカー (TrkA、染色体異常等) から判断して、悪性度が低いと医師によって判断されるものである。本発明の好適な実施の形態では、病期1または2、発症年齢が1歳未満、手術後5年以上再発なく生存し、臨床組織中にN-mycの増幅が認められないものを予後良好型としたが、このような特定の例には限定されない。また、本明細書で使用する「予後不良型」とは、ヒト神経芽細胞腫のうち、腫瘍の進行が認められる状態を指し、N-myc その他腫瘍マーカーから判断して、悪性度が高いと医師によって判断されるものである。本発明の好適な実施の形態では、病期4、発症年齢が1歳以上、手術後3年以内に死亡、臨床組織中にN-mycの増幅が認められたものを予後不良型とし

たが、このような特定の例には限定されない。

【0040】

なお、4 s 期神経芽細胞腫は、上記のような臨床分子生物学的分類に従えば「予後良好型」に分類されるが、本明細書中では便宜上、「予後良好型」とは区別して取り扱う。

【0041】

神経芽細胞腫は、ヒトでは2種類しか知られていない神経細胞そのものの腫瘍の1つであり、そこで発現している遺伝子を解析することは、神経細胞のバイオリロジーを理解する上で非常に有用な知見をもたらすものと考えられる。すなわち、脳や末梢神経から、部位特異的な均質な組織を得ることは極めて困難で、事実上不可能である。一方、神経芽細胞腫は、末梢交感神経細胞に由来するほぼ均一な神経細胞集団（腫瘍化してはいるが）から成り、均質に発現している神経関連遺伝子が得られる可能性が高い。また、神経芽細胞腫は癌であるため、神経発生未熟な段階で発現している重要な遺伝子が多いことも特徴として挙げられる。

【0042】

さらに、神経芽細胞腫は、予後の良好なものと予後の不良なものとが臨床的、生物学的に明瞭に区別される。予後良好型の神経芽細胞腫の癌細胞は、増殖速度が極めて遅く、ある時点から自然退縮を始めることが特徴である。これまでの知見から、この自然退縮では、神経細胞の分化およびアポトーシス（神経細胞死）が起こっており、正常神経細胞の成熟段階で起こる分化とプログラム細胞死と非常によく似た現象であることが分かってきた。従って、この腫瘍で発現している遺伝子を解析することによって、神経の分化やアポトーシスに関連した重要な遺伝子情報を入手できる可能性が極めて高い。

【0043】

上記の有用な遺伝子情報を入手できる遺伝子である本発明の遺伝子およびそれらに由来する本発明の核酸は、4 s 期神経芽細胞腫の臨床組織（以下、4 s とも略称する）に見出されたものであるが、予後良好型の臨床組織（以下、“F (favorable)”とも略称する）および予後不良型の臨床組織（以下、“U F (unfavorable)”とも略称する）でのそれら遺伝子の発現を比較すると以下のような特徴を

有する。

【 0 0 4 4 】

すなわち、前述のようにして得られ、少なくとも部分的にシーケンスした 4 5 2 個の遺伝子をそれぞれのサブセット間での発現パターンに基づいて、分類し 7 つの群にグループ化したところ、次のようになる。

【 0 0 4 5 】

(グループ I)

このグループに属する遺伝子は、その発現 (4 s) が U F と同程度であり、F より低い。さらに、これら遺伝子をサブグループに分類すると、I - 1、I - 2 および I - 3 となる。各サブグループの遺伝子発現パターンについては、表 1 を参照。

【 0 0 4 6 】

I - 1 に属する特定のクローンは、nbla20026(配列番号171), nbla20421(配列番号172), nbla22298(配列番号173), nbla22549(配列番号174)およびnbla23020 (以上、新規遺伝子)である。

【 0 0 4 7 】

I - 2 に属する特定のクローンは、nbla20113, nbla20146(配列番号137), nbla20170(配列番号138), nbla20216(配列番号139), nbla20253, nbla20549, nbla20657(配列番号140), nbla20688(配列番号141), nbla20755(配列番号142), nbla20835, nbla20968, nbla21013(配列番号143), nbla21087, nbla21172(配列番号144), nbla21189, nbla21200(配列番号145), nbla21214, nbla21255(配列番号146), nbla21337, nbla21344, nbla21345(配列番号147), nbla21410(配列番号148), nbla21522(配列番号149), nbla21631(配列番号150), nbla21788(配列番号151), nbla21897(配列番号152), nbla21956, nbla22116(配列番号153), nbla22223(配列番号154), nbla22228, nbla22344(配列番号155), nbla22351, nbla22361, nbla22474, nbla22629, nbla22939(配列番号156), nbla23084(配列番号157), nbla23103(配列番号158), nbla23234(配列番号159), nbla23300(配列番号160), nbla23369(配列番号161), nbla23436(配列番号162), nbla23511(配列番号163), nbla23664(配列番号164), nbla23775, nbla23860(配列番号165), nbla23877(配列番号

166), nbla23998(配列番号167), nbla24043(配列番号168), nbla24182, nbla24285, nbla24402(配列番号169), nbla24434, nbla24460, nbla24762, nbla24821(配列番号170), nbla24893, nbla24973, nbla24986 (以上、新規遺伝子)、nbla20279, nbla20687, nbla20924, nbla21168, nbla21303, nbla21483, nbla21838, nbla21917, nbla22099, nbla22438, nbla23111, nbla23208, nbla24118, nbla24279, nbla24771および nbla24871 (以上、既知遺伝子) である。

【0048】

I-3に属する特定のクローンは、nbla20084(配列番号129), nbla21081(配列番号130), nbla21420(配列番号131), nbla21761, nbla22452(配列番号132), nbla22595(配列番号133), nbla22676(配列番号134), nbla22909(配列番号135), nbla23456, nbla24297, nbla24435(配列番号136), nbla24719 (以上、新規遺伝子)、nbla20117, nbla20238, nbla20904, nbla23293, nbla23297, nbla23311, nbla23589, nbla23629, nbla23862, nbla24133およびnbla24761 (以上、既知遺伝子) である。

【0049】

(グループII)

このグループに属する遺伝子は、その発現(4s)がFと同程度であり、UFより高い。さらに、これら遺伝子をサブグループに分類すると、II-1、II-2およびII-3となる。各サブグループの遺伝子発現パターンについては、表1を参照。

【0050】

II-1に属する特定のクローンは、nbla20365(配列番号117), nbla20378(配列番号118), nbla20511(配列番号119), nbla21039(配列番号120), nbla21107(配列番号121), nbla21367(配列番号122), nbla21790(配列番号123), nbla21855, nbla22253(配列番号124), nbla22355(配列番号125), nbla22704, nbla22832(配列番号126), nbla23394, nbla23512, nbla23755(配列番号127), nbla24084, nbla24376, nbla24549(配列番号128) (以上、新規遺伝子)、nbla20624, nbla22029, nbla22424, nbla22594およびnbla22622 (以上、既知遺伝子) である。

【0051】

II-2 に属する特定のクローンは、nbla20001(配列番号58), nbla20083(配列番号59), nbla20125, nbla20182(配列番号60), nbla20231, nbla20248(配列番号61), nbla20250(配列番号62), nbla20268, nbla20330(配列番号63), nbla20395, nbla23973, nbla23983(配列番号64), nbla24041, nbla24082, nbla24104, nbla24111(配列番号65), nbla24142(配列番号66), nbla24157(配列番号67), nbla24230(配列番号68), nbla24239, nbla20541(配列番号69), nbla20555(配列番号70), nbla20638, nbla20645(配列番号71), nbla20713(配列番号72), nbla20765, nbla20789, nbla20792, nbla20798, nbla21024, nbla24250(配列番号73), nbla24254(配列番号74), nbla24327(配列番号75), nbla24363, nbla24510(配列番号76), nbla24554(配列番号77), nbla24604(配列番号78), nbla24622, nbla24646, nbla24672, nbla21037(配列番号79), nbla21077, nbla21089, nbla21130, nbla21161(配列番号80), nbla21170(配列番号81), nbla21198(配列番号82), nbla21266, nbla21298(配列番号83), nbla21379(配列番号84), nbla24705(配列番号85), nbla24709, nbla24748, nbla24831, nbla24972, nbla21385(配列番号86), nbla21413, nbla21416(配列番号87), nbla21520, nbla21599(配列番号88), nbla21681(配列番号89), nbla21878(配列番号90), nbla21922(配列番号91), nbla21936, nbla22004-2(配列番号92), nbla22004-1(配列番号93), nbla22028, nbla22085(配列番号94), nbla22093, nbla22119(配列番号95), nbla22149(配列番号96), nbla22161(配列番号97), nbla22218, nbla22252(配列番号98), nbla22347(配列番号99), nbla22352(配列番号100), nbla22394(配列番号101), nbla22423(配列番号102), nbla22439(配列番号103), nbla22451, nbla22455, nbla22464, nbla22465, nbla22487, nbla22633(配列番号104), nbla22669, nbla22698(配列番号105), nbla22726, nbla22886, nbla22896(配列番号106), nbla23012, nbla23038, nbla23167(配列番号107), nbla23339(配列番号108), nbla23352(配列番号109), nbla23575(配列番号110), 23592(配列番号111), nbla23601(配列番号112), nbla23630(配列番号113), nbla23718, nbla23719, nbla23754(配列番号114), nbla23892(配列番号115), nbla23951, nbla23956(配列番号116) (以上、新規遺伝子)、nbla20393, nbla20423, nbla20510, nbla20833, nbla20931, nbla20943, nbla21258, nbla21268, nbla21273, nbla21412, nbla21578, nbla21614, nbla21624, nbla2165

5, nbla21670, nbla21787, nbla21954, nbla21979, nbla22043, nbla22137, nbla22192, nbla22325, nbla22327, nbla22337, nbla22482, nbla22763, nbla22788, nbla22839, nbla22851, nbla22935, nbla22937, nbla23238, nbla23327, nbla23360, nbla23519, nbla23553, nbla23554, nbla23683, nbla23812, nbla23823, nbla23849, nbla23882, nbla23910, nbla24064, nbla24405, nbla24897およびnbla24913 (以上、既知遺伝子) である。

【 0 0 5 2 】

II-3 に属する特定のクローンは、nbla20134, nbla20181, nbla20264 (配列番号31), nbla20269 (配列番号32), nbla20276, nbla20406 (配列番号33), nbla20709, nbla20782, nbla20788, nbla20949 (配列番号34), nbla21046, nbla21122, nbla21211, nbla21233, nbla21251 (配列番号35), nbla21334 (配列番号36), nbla21356 (配列番号37), nbla21375, nbla21418 (配列番号38), nbla21480 (配列番号39), nbla21509 (配列番号40), nbla21524, nbla21527 (配列番号41), nbla21551 (配列番号42), nbla21735 (配列番号43), nbla21843, nbla21934, nbla22153, nbla22247 (配列番号44), nbla22382, nbla22477 (配列番号45), nbla22571, nbla22639 (配列番号46), nbla22789, nbla23060, nbla23174 (配列番号47), nbla23198 (配列番号48), nbla23218, nbla23328 (配列番号49), nbla23420 (配列番号50), nbla23483 (配列番号51), nbla23545, nbla23653, nbla23666, nbla23760, nbla23808 (配列番号52), nbla23830, nbla23851 (配列番号53), nbla23942, nbla24011 (配列番号54), nbla24131, nbla24235 (配列番号55), nbla24556 (配列番号56), nbla24800 (配列番号57), nbla24908 (以上、新規遺伝子)、nbla20133, nbla20263, nbla20723, nbla20748, nbla20915, nbla21016, nbla21034, nbla21067, nbla21167, nbla21319, nbla21331, nbla21516, nbla21682, nbla21691, nbla21822, nbla21976-2, nbla21977, nbla22159, nbla22168, 22215-1, nbla22244, nbla22263, nbla22548, nbla23033, nbla23231, nbla23284, nbla23329-1, nbla23384, nbla23556, nbla23674, nbla23879-2, nbla24098, nbla24329, nbla24334, nbla24439-1, nbla24443, nbla24507, nbla24836, nbla24958およびnbla24989 (以上、既知遺伝子) である。

【 0 0 5 3 】

(グループIII)

このグループに属する遺伝子は、その発現 (4 s) が F と同程度であり、U F より低い。さらに、これら遺伝子をサブグループに分類すると、III-1、III-2 および III-3 となる。各サブグループの遺伝子発現パターンについては、表 1 を参照。

【0054】

III-1 に属する特定のクローンは、nbla20874 (新規遺伝子) および nbla23262 (既知遺伝子) である。

【0055】

III-2 に属する特定のクローンは、nbla20604, nbla21226, nbla21908 (配列番号27), nbla21928, nbla22027 (配列番号28), nbla22082 (配列番号29), nbla22643, nbla23303 (配列番号30), nbla23649, nbla24468 (以上、新規遺伝子)、nbla20141, nbla20446, nbla21538, nbla21558, nbla21623, nbla21969, nbla22219, nbla23272, nbla23307 および nbla24117 (以上、既知遺伝子) である。

【0056】

III-3 に属する特定のクローンは、nbla20578 (配列番号26), nbla21212 (以上、新規遺伝子)、nbla23478, nbla23896 および nbla24920 (以上、既知遺伝子) である。

【0057】

(グループIV)

このグループに属する遺伝子は、その発現 (4 s) が U F と同程度であり、F より高い ($F < 4s = UF$)。このグループに属する特定のクローンは、nbla23899 (配列番号25) および nbla24526 (以上、新規遺伝子) である。

【0058】

(グループV)

このグループに属する遺伝子は、その発現 (4 s) が F より低く、U F より高い。さらに、これら遺伝子をサブグループに分類すると、V-1、V-2、V-3、V-4 および V-5 となる。各サブグループの遺伝子発現パターンについては、表 1 を参照。

【0059】

V-1に属する特定のクローンは、nbla22031(既知)である。V-2に属する特定のクローンは、nbla22305(既知)である。

【0060】

V-3に属する特定のクローンは、nbla20123(配列番号17), nbla20382(配列番号18), nbla20660(配列番号19), nbla20666(配列番号20), nbla21239(配列番号21), nbla21729(配列番号22), nbla21831(配列番号23), nbla22826(配列番号24), nbla24521(以上、新規遺伝子)、nbla20235およびnbla22607(以上、既知遺伝子)である。

【0061】

V-4に属する特定のクローンは、nbla20787(配列番号15), nbla22284(配列番号16)およびnbla24756(以上、新規遺伝子)である。

【0062】

V-5に属する特定のクローンは、nbla24348およびnbla24686(以上、新規遺伝子)である。

【0063】

(グループVI)

このグループに属する遺伝子は、その発現(4s)がFおよびUFより低いか、またはFおよびUFより高い。さらに、これら遺伝子をサブグループに分類すると、VI-1、VI-2、VI-3、VI-4、VI-5、VI-6、VI-7およびVI-8となる。各サブグループの遺伝子発現パターンについては、表1を参照。

【0064】

VI-1に属する特定のクローンは、nbla21297(配列番号14)(新規遺伝子)およびnbla22443(既知遺伝子)である。

【0065】

VI-2に属する特定のクローンは、nbla20211, nbla20469, nbla21250, nbla22182(配列番号12), nbla22761, nbla23256(配列番号13), nbla23631, nbla23711, nbla24532, nbla24951(以上、新規遺伝子)、nbla21750, nbla22129, nbla22808, nbla23064およびnbla23358(以上、既知遺伝子)である。

【0066】

VI-3に属する特定のクローンは、nbla20226(配列番号11)(新規遺伝子)である。

【0067】

VI-4に属する特定のクローンは、nbla21650(配列番号7), nbla22094(配列番号8), nbla22739(配列番号9)およびnbla23525(配列番号10)(以上、新規遺伝子)である。

【0068】

VI-5に属する特定のクローンは、nbla23701(配列番号5)およびnbla23890(配列番号6)(以上、新規遺伝子)である。

【0069】

VI-6に属する特定のクローンは、nbla20087(既知遺伝子)である。

【0070】

VI-7に属する特定のクローンは、nbla22689(配列番号2), nbla22968, nbla24079, nbla24135(配列番号3)およびnbla24350(配列番号4)(以上、新規遺伝子)である。

【0071】

VI-8に属する特定のクローンは、nbla22256(新規遺伝子)である。

【0072】

(グループVII)

このグループに属する遺伝子(1個のみ)は、4sでのみ発現している。その特定のクローンは、nbla22420(配列番号1)(新規遺伝子)である。

【0073】

前記それぞれのグループについて、遺伝子群を新規な遺伝子と、既知の遺伝子に分け、まとめたものが表1である。

【表1】

グループ	発現パターン	新規遺伝子	既知遺伝子	計
I-1	$F \gg 4s = UF$	5	0	5
I-2	$F > 4s = UF$	59	16	75
I-3	$F \geq 4s = UF$	12	11	23
II-1	$F = 4s \gg UF$	18	5	23
II-2	$F = 4s > UF$	105	47	152
II-3	$F = 4s \geq UF$	55	40	95
III-1	$F = 4s \ll UF$	1	1	2
III-2	$F = 4s < UF$	10	10	20
III-3	$F = 4s \leq UF$	2	3	5
IV	$F < 4s = UF$	2	0	2
V-1	$F > 4s \gg UF$	0	1	1
V-2	$F \geq 4s \gg UF$	0	1	1
V-3	$F > 4s > UF$	9	2	11
V-4	$F \geq 4s > UF$	3	0	3
V-5	$F \geq 4s \geq UF$	2	0	2
VI-1	$F \gg 4s < UF$	1	1	2
VI-2	$F > 4s < UF$	10	5	15
VI-3	$F > 4s \leq UF$	1	0	1
VI-4	$F \geq 4s \leq UF$	4	0	4
VI-5	$F < 4s \gg UF$	2	0	2
VI-6	$F \leq 4s \gg UF$	0	1	1
VI-7	$F < 4s > UF$	5	0	5
VI-8	$F \leq 4s \geq UF$	1	0	1
VII	4s のみ	1	0	1
クローン総数		308	144	452

なお、表中および上記分類において、「=」は遺伝子発現量がサブセット間でほぼ等しいことを示す。

【0074】

例えば、グループVIに属する遺伝子群は、4 s 期神経芽細胞腫における遺伝子発現量と、予後良好型および予後不良型の臨床組織における同一遺伝子の遺伝子発現量を比較すると、4 s 期神経芽細胞腫において特異的である（すなわち、いずれよりもかなり高いか、或いはかなり低い）。従って、これらの遺伝子の少なくともひとつの存在を臨床組織サンプルに検出すれば、4 s 期神経芽細胞腫である可能性が高いとの判定ができる。

【0075】

また、グループVIIに属する遺伝子は、4 s 期神経芽細胞腫の臨床組織においてのみ、検出されている。従って、この遺伝子の存在を臨床組織サンプルに検出すれば、4 s 期神経芽細胞腫である可能性が高いとの判定ができることになる。

【0076】

さらに、残りのグループに属する遺伝子群も、4 s 期神経芽細胞腫における、遺伝子発現量と、予後良好型および予後不良型の臨床組織における同一遺伝子の遺伝子発現量を比較すると、上記のような発現パターンが見出される。従って、これらの遺伝子の発現パターンを複数個、検出して、それらを解析すれば、検定する臨床組織サンプルが4 s 期神経芽細胞腫であるかどうかの判定ができる。特に、この目的で本発明の核酸を使用するとき、後述の核酸マイクロアレイを作製して、前記判定に供することが好ましい。

【0077】

このように、本発明の核酸は神経芽細胞腫の予後の良不良を診断する腫瘍マーカーとして有用である。すなわち、本発明は、ヒト神経芽細胞腫の予後およびそれに関連する様々な遺伝子情報を以下の手段により提供可能とする。

【0078】

(1) ハイブリダイゼーションに用いるプローブ

本発明の1つの実施の形態に従えば、本発明の核酸をハイブリダイゼーションのプローブ（すなわち、本発明の核酸プローブ）として使用することによって、神経芽細胞腫で発現している本発明の遺伝子を検出することが可能である。さらに、本発明の核酸をハイブリダイゼーションのプローブとして使用し、様々な腫

瘍、正常組織における遺伝子発現を調べることによって、該遺伝子発現の分布を同定することも可能である。

【0079】

本発明の核酸をハイブリダイゼーションのプロープとして使用する場合、ハイブリダイゼーション方法自身については特に限定されない。好適な方法としては、例えばノザンハイブリダイゼーション、サザンハイブリダイゼーション、コロニーハイブリダイゼーション、ドットハイブリダイゼーション、Fluorescence in situ hybridization (FISH)、in situ hybridization (ISH)、DNAチップ法、マイクロアレイ法、などが挙げられる。

【0080】

前記ハイブリダイゼーションの1つの応用例として、本発明の核酸をノザンハイブリダイゼーションのプロープとして用い、検定する臨床組織サンプル中においてmRNAの長さを測定することや、遺伝子発現を定量的に検出することが可能である。

【0081】

また、別の応用例として、本発明の核酸をサザンハイブリダイゼーションのプロープとして用い、検定する臨床組織サンプルのゲノムDNA中の、該DNA配列の有無を検出することが可能である。

【0082】

さらに別の応用例として、本発明の核酸をFISH法のプロープとして用い、本発明の遺伝子の染色体上の位置を同定することも可能である。

【0083】

さらに別の応用例として、本発明の核酸をISH法のプロープとして用い、本発明の遺伝子の発現の組織分布を同定することも可能である。

【0084】

本発明の核酸をハイブリダイゼーション用プロープとして使用する場合、少なくとも20個の塩基長が必要であり、本発明の核酸のうち、20個以上の連続した塩基からなる核酸が好ましく用いられる。より好ましくは、40個以上の連続した塩基からなる核酸が用いられる。特に好ましくは、60個以上の連続した塩

基からなる核酸が用いられる。さらに、配列表の配列番号1～174に記載の核酸配列の全長からなる核酸を用いてもよい。

【0085】

当業者にとって、上記各種のハイブリダイゼーションにおける核酸プローブ技法は周知であり、例えば、個々の塩基長を有する本発明の核酸プローブと、目的とするポリヌクレオチドとの適当なハイブリダイズ条件は容易に決定することができる。種々の塩基長を含むプローブに対し至適であるハイブリダイズ条件を得るためのかかる操作は、当業者では周知であり、例えばサンプルブックら、モレキュラー・クローニング：ア・ラボラトリー・マニュアル (Molecular Cloning: A laboratory manual) (前掲) を参照して、行えばよい。

【0086】

好ましくは、本発明の核酸プローブは、容易に検出されるように標識される。検出可能な標識は、目視によって、または機器を用いるかのいずれかによって検出され得るいかなる種類、元素または化合物であってもよい。通常使用される検出可能な標識としては、放射性同位元素、アビジンまたはビオチン、蛍光物質 (FITC または ロードミン等) が挙げられる。前記放射性同位元素は、 ^{32}P 、 ^{14}C 、 ^{125}I 、 ^3H 、 ^{35}S 等である。また、ビオチン標識ヌクレオチドは、ニックトランスレーション、化学的または酵素的手段によって、核酸に組み込むことができる。ビオチン標識されたプローブは、アビジン/ストレプトアビジン、蛍光標識、酵素、金コロイド複合体等などの標識手段を使用したハイブリダイゼーション後に検出される。また、本発明の核酸プローブは、タンパク質と結合させることによって標識されてもよい。その目的で、例えば放射性または蛍光ヒストン一本鎖結合タンパク質が使用される。このようにして、適当に標識されたプローブは、本発明の診断剤を構成する。

【0087】

(2) PCR に用いるプライマー

本発明の遺伝子を検出するには上記のハイブリダイゼーション法の他に、本発明の核酸に含まれる任意の核酸 (DNA) 配列からプライマーを設計して、Polymerase Chain Reaction (PCR) 法を用いることにより可能である。例えば、

検定する臨床組織サンプルから mRNA を抽出し、RT-PCR 法により遺伝子発現を半定量的に測定することが可能である。このような方法は、当業者にとって周知の方法に従って行われるが、例えば、サンプルックら、モレキュラー・クローニング：ア・ラボラトリー・マニュアル (Molecular Cloning: A laboratory manual) (前掲)、および遺伝子病入門 (高久史磨著：南江堂) が参照される。

【0088】

本発明の核酸 (DNA) を PCR 用プライマー (すなわち、本発明のプライマー) として使用する場合、10 ないし 60 個の塩基長が必要であり、本発明に係る核酸配列の一部であって、10 ないし 60 個の連続した塩基を有する核酸が好ましく用いられる。より好ましくは、15 ないし 30 個の塩基を有するものが用いられる。また一般的には、プライマー配列中の GC 含量が 40 ないし 60% のものが好ましい。さらに、増幅に用いる 2 つのプライマー間の T_m 値に差がないことが望まれる。また、プライマーの 3' 末端でアニールせず、プライマー内で 2 次構造をとらないことも望ましい。

【0089】

(3) 遺伝子のスクリーニング

本発明の核酸を使用することによって、神経芽細胞腫のみならず様々な組織や細胞で発現している本発明の遺伝子の発現 (またはその分布) を検出することが可能である。これは例えば、本発明の核酸を上記のようにハイブリダイゼーションのプロブ、または PCR のプライマーとして使用することによって、可能となる。

【0090】

また、DNA チップ、核酸マイクロアレイ等を用いても遺伝子の発現分布を検出することが可能である。すなわち、本発明の核酸を直接、前記チップ、アレイ上に張り付けることが出来る。チップ、アレイに張り付けるために、高精度分注機でかかる核酸等 (DNA) を基板にスポットする方法が知られている (例えば、米国特許第 5807522 号を参照)。そこに臨床組織サンプルから抽出した mRNA を蛍光物質などで標識し、ハイブリダイズさせ、その遺伝子がどの様な

組織の細胞で高発現しているかを解析することが可能である。またチップ、アレイ上に張り付けるDNAは、本発明の核酸またはその断片をプローブとして用いたPCRの反応産物であってもよい。別法として、本発明の核酸断片（DNA断片）を基板上で直接合成してDNAチップ若しくはアレイとすることもできる（例えば、米国特許第5424186号を参照）。

【0091】

（4）DNAのクローニング

本発明の核酸を使用することによってヒト神経芽細胞腫において発現している遺伝子をクローニングすることが可能である。例えば、本発明の核酸をノザンハイブリダイゼーションのプローブ、コロニーハイブリダイゼーションのプローブまたはPCRのプライマーとして使用し、本発明の遺伝子をクローニングすることが可能である。クローニング可能な遺伝子としては特に、予後不良型の神経芽細胞腫と予後不良型の神経芽細胞腫で発現量に差がある遺伝子、4s期神経芽細胞腫で発現する遺伝子、他の組織や癌細胞での発現様式とは異なって発現している遺伝子、細胞周期依存的に発現している遺伝子、神経分化に伴って誘導される遺伝子、癌遺伝子または癌抑制遺伝子によって発現が制御される遺伝子等が挙げられる。

【0092】

（5）腫瘍の予後診断の方法およびそのために使用可能な腫瘍マーカー

上述のように本発明の遺伝子は、4s期神経芽細胞腫（予後良好型および予後不良型の神経芽細胞腫を含めて）において発現が見出された。そこで、本発明の核酸をハイブリダイゼーションのプローブ或いはPCRのプライマーとして使用し、被験者から採取した、検定する臨床組織サンプル中で、前記遺伝子の発現パターンを調べることにより予後診断（4s期神経芽細胞腫の判定）が行える。遺伝子の検出方法としては、前述のノーザンブロットハイブリダイゼーション法、インサイチュハイブリダイゼーション法、およびRT-PCR法等が挙げられる。

【0093】

ハイブリダイゼーション法を用いるとき、検出する臨床組織サンプル中で前記

核酸プローブとハイブリダイズする核酸の量を対照サンプル（例えば、予後良好型および予後不良型の神経芽細胞腫からの臨床組織）と比較して、遺伝子発現パターンを決定する。このようにして遺伝子発現パターンを検出するのに使用したそれぞれの核酸について、例えば、表 1 に記載の発現パターンと比較、解析して、予後診断できる。この目的では、前記の核酸マイクロアレイの使用が望ましい。また、RT-PCR 法を用いるとき、サンプルから mRNA を抽出し、これを DNA に逆転写して、前記プライマーにより増幅する RT-PCR 法を用いて、遺伝子発現を半定量的に測定する。それから前記と同様にして、予後診断できる。この目的のためには、該プライマーを必須成分として一組含有する診断キットを用いることが好ましい。該診断キットは、プライマー成分以外に、PCR 用の緩衝液、洗浄液、および酵素等の公知の成分を含む。

【0094】

(6) アンチセンスオリゴヌクレオチド

本発明の別の実施の形態に従えば、本発明の核酸に対するアンチセンスオリゴヌクレオチドが提供される。前記アンチセンスオリゴヌクレオチドは、本発明の核酸にハイブリダイズすることが可能であり、アンチセンス DNA とアンチセンス RNA とを含む。アンチセンス DNA は、DNA から mRNA への転写を阻害し、アンチセンス RNA は、mRNA の翻訳を阻害する。このようなアンチセンスオリゴヌクレオチドは、自動合成機を使用して、または本発明の核酸を鋳型とする PCR 法により合成できる。さらに、該アンチセンスオリゴヌクレオチドは、DNA や mRNA との結合力、組織選択性、細胞透過性、ヌクレアーゼ耐性、細胞内安定性が高められたアンチセンスオリゴヌクレオチド誘導体をも包含する。このような誘導体は、公知のアンチセンス技術を用いて、合成することができる。

【0095】

mRNA の翻訳開始コドン付近、リボソーム結合部位、キャッピング部位、スプライス部位の配列に相補的な配列を有するアンチセンスオリゴヌクレオチドは、該 RNA の合成を阻止することができ、特に遺伝子の発現抑制効果が高い。従って、本発明は、かかるアンチセンスオリゴヌクレオチドを好適に包含する。

【0096】

(7) 遺伝子治療

本発明の別の実施の形態に従えば、遺伝子治療に用いられる治療用遺伝子をコードする核酸配列が提供される。そこで、本発明の核酸を遺伝子運搬に使用されるベクターに導入して、任意の発現プロモーターにより導入遺伝子（本発明の遺伝子）を発現させ、遺伝子治療に用いることができる。

【0097】

1. ベクター

導入されうるウイルスベクターは、DNAまたはRNAウイルスをもとに作製できる。このようなベクターは、MoMLVベクター、ヘルペスウイルスベクター、アデノウイルスベクター、AAVベクター、HIVベクター、SIVベクター、センダイウイルスベクター等のいかなるウイルスベクターであってもよい。また、ウイルスベクターの構成タンパク質群のうち1つ以上を、異種ウイルスの構成タンパク質に置換する、または、遺伝子情報を構成する核酸配列のうち一部を異種ウイルスの核酸配列に置換する、シュードタイプ型のウイルスベクターも本発明に使用できる。例えば、HIVの外皮タンパク質であるEnvタンパク質を、小水痘性口内炎ウイルス (vesicular stomatitis Virus: VSV) の外皮タンパク質であるVSV-Gタンパク質に置換したシュードタイプウイルスベクターが挙げられる [ナルジニ・エルら (Naldini L.), サイエンス (Science), 米国, 1996年, 第272巻, p. 263]。さらに、治療効果を持つウイルスであれば、ヒト以外の宿主域を持つウイルスもウイルスベクターとして使用可能である。ウイルス以外のベクターとしてはリン酸カルシウムと核酸の複合体、リポソーム、カチオン脂質複合体、センダイウイルスリポソーム、ポリカチオンを主鎖とする高分子キャリアー等が使用可能である。さらに遺伝子導入系としてはエレクトロポレーション、遺伝子銃等も使用可能である。

【0098】

2. 発現プロモーター

さらに、治療用遺伝子に用いられる発現カセットは、標的細胞内で遺伝子を発現させることができるものであれば、特に制限されることなくいかなるものでも

用いることができる。当業者はそのような発現カセットを容易に選択することができる。好ましくは、動物由来の細胞内で遺伝子発現が可能な発現カセットであり、より好ましくは、哺乳類由来の細胞内で遺伝子発現が可能な発現カセットであり、特に好ましくは、ヒト由来の細胞内で遺伝子発現が可能な発現カセットである。発現カセットに用いられる遺伝子プロモーターは、例えばアデノウイルス、サイトメガロウイルス、ヒト免疫不全ウイルス、シミアンウイルス 40、ラウス肉腫ウイルス、単純ヘルペスウイルス、マウス白血病ウイルス、シンビスウイルス、A型肝炎ウイルス、B型肝炎ウイルス、C型肝炎ウイルス、パピローマウイルス、ヒトT細胞白血病ウイルス、インフルエンザウイルス、日本脳炎ウイルス、JCウイルス、パルボウイルスB19、ポリオウイルス等のウイルス由来のプロモーター、アルブミン、SR α 、熱ショック蛋白、エロンゲーション因子等の哺乳類由来のプロモーター、CAGプロモーター等のキメラ型プロモーター、テトラサイクリン、ステロイド等によって発現が誘導されるプロモーターを含む。

【0099】

3. 医薬品

遺伝子治療に用いる医薬品は、上記のような治療用にデザインされた薬物遺伝子を含む組換えウイルスベクターとして調製される。より具体的に言えば、本発明の遺伝子を含む組換えウイルスベクターを、水、生理食塩水、等張化した緩衝液等の適当な溶媒に溶解することで調製できる。その際、ポリエチレングリコール、グルコース、各種アミノ酸、コラーゲン、アルブミン等を保護材として添加しても調製可能である。

【0100】

4. 投与法、投与量

上記医薬品の生体への投与の方法については特に制限はない。例えば非経口的投与（注射投与など）することにより好ましく実施できる。その医薬品の使用量は、その使用方法、使用目的等により異なり、当業者は容易に適宜選択および最適化することが可能である。例えば、注射投与して用いる場合には、1日量約0.1 $\mu\text{g}/\text{kg}$ ~ 1,000 mg/kg を投与するのが好ましく、より好ましく

は、1日量約 $1\mu\text{g}/\text{kg} \sim 100\text{mg}/\text{kg}$ である。

【0101】

以下、実施例に即してさらに詳しく説明するが、本発明の技術的範囲はこれらの例に限定されるものではない。

【0102】

【実施例】

以下、実施例に基づいて本発明をより具体的に説明するが、本発明は以下の実施例に限定されるものではない。

【0103】

(製造例1) 神経芽細胞腫からの cDNA ライブラリーの作製

1. サンプル入手

ヒト神経芽細胞腫 (4 s 期) の臨床組織サンプルを手術摘出直後に準無菌的に凍結し、その後 -80°C に保存した。

【0104】

2. mRNA の調製

1 に記載のサンプル 2 ~ 3 g を Total RNA Extraction Kit (QIGEN 社製) で処理し、トータル RNA を抽出した。抽出したトータル RNA をオリゴ dT セルロースカラム (Collaborative 社製) を用いて、poly A 構造を有する mRNA プールに精製した。さらに、以下の手順に従い、オリゴキャッピング法 [Y. Suzuki ら、ジーン (Gene), 米国, 1997 年, 第 200 巻, p. 149-156] を用いて cDNA ライブラリーを調製した。

【0105】

3. mRNA の脱リン酸化

上記 2 において調製した $100 \sim 200\mu\text{g}$ の mRNA プールを $67.3\mu\text{l}$ の 0.1% ジエチルピロカーボネート (DEPC) を含む滅菌超純水 (DEPC-H₂O) に溶解させ、 $20\mu\text{l}$ の 5x BAP バッファー [Tris-HCl (500mM, pH=7.0) /メルカプトエタノール (50mM)]、 $2.7\mu\text{l}$ の RNasin (40 unit/ μl : Promega 社製)、 $10\mu\text{l}$ の BAP (0.25 unit/ μl 、バクテリア由来アルカリフォスファターゼ: 宝

酒造社製)を加えた。この混合液を37℃で1時間反応させ、mRNAの5'末端の脱リン酸化処理を行った。その後、フェノール・クロロホルム処理を2回行い、最後にエタノール沈殿により、脱リン酸化mRNAプールを精製した。

【0106】

4. 脱リン酸化mRNAの脱キャップ処理

上記3において調製した脱リン酸化mRNAプールの全量を75.3 μ lの0.1%DEPCを含む滅菌超純水に溶解させ、20 μ lの5xTAPバッファー[酢酸ナトリウム(250mM、pH=5.5)/メルカプトエタノール(50mM)、EDTA(5mM、pH=8.0)]、2.7 μ lのRNasin(40unit/ μ l)、2 μ lのTAP(Tobacco Acid pyrophosphatase:20unit/ μ l)]を加えた。この混合液を37℃で1時間反応させ、脱リン酸化mRNAの5'末端の脱キャップ処理を行った。この際、キャップ構造を持たない不完全長の脱リン酸化mRNAは、脱キャップ処理されず5'末端は脱リン酸化された状態に留まった。その後、フェノール・クロロホルム処理、エタノール沈殿により、脱キャップmRNAプールを精製した。

【0107】

5. オリゴキャップmRNAの調製

上記4において調製した脱キャップmRNAプールの全量を11 μ lの0.1%DEPCを含む滅菌超純水に溶解させ、4 μ lの5'-オリゴRNA(5'-AGCAUCGAGUCGGCCUUGGCCUACUGG-3':配列番号1079;100ng/ μ l)、10 μ lの10xligationバッファー[Tris-HCl(500mM、pH=7.0)/メルカプトエタノール(100mM)]、10 μ lの塩化マグネシウム(50mM)、2.5 μ lのATP(24mM)、2.5 μ lのRNasin(40unit/ μ l)、10 μ lのT4 RNA ligase(25unit/ μ l:宝酒造社製)、50 μ lのポリエチレングリコール(50%w/v、PEG8000:シグマ社製)を加えた。この混合液を20℃で3時間反応させ、脱キャップmRNAの5'末端に5'-オリゴRNAを連結した。この際、キャップ構造を持たない不完全長の脱リン酸化mRNAは、5'-オリゴRNAが連結されない。その後、フェノール・クロロホルム処理、エタノール沈殿に

より、オリゴキャップmRNAプールを精製した。

【0108】

6. オリゴキャップmRNAからのDNA除去

上記5において調製したオリゴキャップmRNAプールを70.3 μ lの0.1%DEPCを含む滅菌超純水に溶解させ、4 μ lのTris-HCl (1M、pH=7.0)、5.0 μ lのDTT (0.1M)、16 μ lの塩化マグネシウム (50mM)、2.7 μ lのRNasin (40 unit/ μ l)、2 μ lのDNase I (5 unit/ μ l:宝酒造社製)を加えた。この混合液を37℃で10分間反応させ、余分なDNAを分解した。その後、フェノール・クロロホルム処理、エタノール沈殿、カラム精製 (S-400HR:ファルマシアバイオテック社製)により、DNA (-) オリゴキャップmRNAプールを精製した。

【0109】

7. First Strand cDNAの調製

上記6において調製したDNA (-) オリゴキャップmRNAプールを、Super Script II (ライフテックオリエンタル社製キット)を用いて逆転写し、First Strand cDNAプールを得た。

【0110】

DNA (-) オリゴキャップmRNAプールを21 μ lの滅菌蒸留水に溶解させ、10 μ lの10xFirst strandバッファー (キット付属品)、8 μ lのdNTPmix (5mM、キット付属品)、6 μ lのDTT (0.1M、キット付属品)、2.5 μ lのオリゴ-dTアダプタープライマー (5 pmol/ μ l、5'-GCGGCTGAAGACGGCCTATGTGGCCTTTTTTTTTTTTTTTT-3'配列番号1080)、2.0 μ lのRNasin (40 unit/ μ l)、2 μ lのSuper Script II RTase (キット付属品)を加えた。この混合液を42℃で3時間反応させ、逆転写反応を行った。その後、フェノール・クロロホルム処理、アルカリ処理、中和処理にて全てのRNAを分解し、エタノール沈殿で精製した。

【0111】

8. Second Strand cDNAの調製

上記7において調製したFirst Strand cDNAプールを、Gene Amp

(パーキンエルマー社製キット)を用いて、PCR増幅した。First Strand cDNA プールを $52.4 \mu\text{l}$ の滅菌蒸留水に溶解させ、 $30 \mu\text{l}$ の $3 \times \text{Reaction}$ バッファー (キット付属品)、 $8 \mu\text{l}$ の dNTP mix (2.5 mM、キット付属品)、 $4.4 \mu\text{l}$ の酢酸マグネシウム (25 mM、キット付属品)、 $1.6 \mu\text{l}$ のプライマー F ($10 \text{ pmol}/\mu\text{l}$ 、5'-AGCATCGAGTCGGCC TTGTTG-3' 配列番号 1081)、 $1.6 \mu\text{l}$ のプライマー R ($10 \text{ pmol}/\mu\text{l}$ 、5'-GCGCTGAAGACGGCCTATGT-3' 配列番号 1082)、 $2 \mu\text{l}$ の rTth (キット付属品)を加えた。この混合液に、 $100 \mu\text{l}$ のミネラルオイルを静かに加え重層した。この反応液を 94°C で 5 分間変性させた後、 94°C 、1 分間、 52°C 、1 分間、 72°C 、10 分間を 1 サイクルとして 12 サイクル繰り返し、さらに 72°C で 10 分間放置し、PCR 反応を行った。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し、Second Strand cDNA プールを得た。

9. Second Strand cDNA の Sfi I 処理

上記 8 において調製した Second Strand cDNA プールを $87 \mu\text{l}$ の滅菌蒸留水に溶解させ、 $10 \times \text{NEB}$ バッファー (NEB 社製)、 $100 \times \text{BSA}$ (ウシ血清アルブミン、NEB 社製)、 $2 \mu\text{l}$ の Sfi I (制限酵素、 $20 \text{ unit}/\mu\text{l}$ 、NEB 社製)を加えた。この混合液を 50°C で一晩反応させ、Sfi I による制限酵素処理を行った。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し、両末端が Sfi I 処理された cDNA プールを得た。

【0112】

10. Sfi I 処理された cDNA のサイズ分画

上記 9 において調製した Sfi I 処理された cDNA プールを 1% のアガロースゲルで電気泳動し、2 kb 以上の分画を Gene clean II (Bio 101 社製)を用いて精製した。精製した cDNA プールは $100 \mu\text{l}$ の滅菌蒸留水に溶解させ、 37°C で 6 時間放置した。その後、フェノール・クロロホルム処理、エタノール沈殿で精製し、長鎖 cDNA プールを得た。

【0113】

11. cDNA ライブラリー

上記10において調製した長鎖cDNAプールをDNA Ligation kit ver.1 (宝酒造社製キット) を用いてクローニングベクターであるpME18S-FL3 (東京大学医科学研究所 菅野純夫教授より供与) にライゲーションを行った。長鎖cDNAプールを8 μ lの滅菌蒸留水に溶解し、あらかじめ制限酵素DraI IIで処理した1 μ lのpME18S-FL3、80 μ lのSolution A (キット付属品)、10 μ lのSolution B (キット付属品) を加え、16℃で3時間反応させた。その後、フェノール・クロロホルム処理、エタノール沈殿で精製しcDNAライブラリーを得た。

【0114】

(実施例1) 大腸菌へのトランスフォーメーション

1. クローニング

製造例1の12で調製したcDNAライブラリーを大腸菌 (TOP-10、Invitrogen社製) にトランスフォーメーションした。すなわち、cDNAライブラリーを10 μ lの滅菌蒸留水に溶解し、TOP-10に混合した。その後、氷上にて30分間、40℃で1分間、氷上で5分間インキュベートした。500 μ lのSOB培地を加え、37℃で60分間振盪培養した。アンピシリンを含む寒天培地上に適量ずつ播種し、37℃で一昼夜培養して、大腸菌クローンを得た。ここで、5075個のクローンを無作為にピックアップした。

【0115】

2. 大腸菌クローンの保存 (グリセロールストックの調製)

上記1において得られた寒天培地上の各大腸菌クローンを、爪楊枝にて拾い上げ、96穴プレートに準備した120 μ lのLB培地中に懸濁させた。この96穴プレートを37℃で一晩静置し、大腸菌の培養を行った。その後、60%グリセロール溶液を72 μ l加え、-20℃で保存した (グリセロールストック)。

【0116】

(実施例2) 核酸配列決定

1. プラスミドの調製

実施例1の2で調製した10 μ lのグリセロールストックを15 mlの遠心チューブに移し、3 mlのLB培地、50 μ g/mlのアンピシリンを加え、37

℃で一晩振盪し、大腸菌の培養を行った。その後、QIA Prep Spin Miniprep Kit (QIAGEN社製) を用いて大腸菌からプラスミドDNAを抽出、精製した。

【0117】

2. 両末端シーケンスの解析

上記1において調製したプラスミドDNAをDNA Sequencing Kit (ABI社製キット) を用いて両末端のシーケンスを決定した。600 ngのプラスミドDNA、8 μ lのプレミックス (キット付属品)、3.2 pmolのプライマーを混合し、滅菌蒸留水で合計20 μ lになるように調製した。この混合液を96℃で2分間変性させた後、96℃、10秒間、50℃、5秒間、60℃、4分間を1サイクルとして25サイクル繰り返し反応を行った。その後エタノール沈殿で精製した。変性条件下でポリアクリルアミドゲルにて電気泳動を行い、ABI 377 (ABI社製) を用いて配列決定を行った。

【0118】

(実施例3) データベースを用いるホモロジー検索

実施例2において両末端シーケンスを解析して得られたサンプルのDNA配列情報についてインターネットを介したDNA配列のホモロジー検索を行った。検索にはNCBI (National Center of Biotechnology Information USA, <http://www.ncbi.nlm.nih.gov/BLAST>) のBLASTを用いた。BLASTサーチのソフトとして、DYNACLUSt Ver.4.0 (DYNACOM社) を使用した。ホモロジー検索の結果、約2700個の遺伝子を同定した。これらの遺伝子を分類し、RepeatMaskerソフトを使用して反復配列を取り除いたところ、1598個の遺伝子が得られた。そのうち、新規な遺伝子は、963個であり、既知の遺伝子は635個であった。

【0119】

これらの遺伝子のうち、新規なもの308個については、シーケンスできたものに関して、配列表にそれらの部分解読配列を示してある。

【0120】

(実施例4) 半定量的RT-PCRによる遺伝子発現の比較

1. サンプル入手

ヒト神経芽細胞腫（4 s 期）の臨床組織サンプルを手術摘出直後に準無菌的に凍結し、その後 -80°C に保存した。このようなサンプルを8検体用意した。同様に、予後良好型および予後不良型のヒト神経芽細胞腫の臨床組織サンプルを各12検体ずつ用意した。

【0121】

予後良好型および予後不良型の神経芽細胞腫サンプルについては、予後の検定を以下の指標をもとに行ったものである。

予後良好型：

- ・病期1または2
- ・発症年齢が1歳未満
- ・手術後5年以上再発なく生存
- ・N-mycの増幅なし

予後不良型：

- ・病期4
- ・発症年齢が1歳以上
- ・手術後3年以内に死亡
- ・N-myc増幅あり

【0122】

2. ディファレンシャルスクリーニング

各検体の半定量的RT-PCRは以下の方法により実施した。

a) 逆転写（RT）反応

【0123】

検体からのRNAをSuperScript II reverse transcriptase（GIBCO社製）を用いて、cDNAに逆転写した。すなわち、トータルRNA $20\mu\text{g}$ 、 $8\mu\text{l}$ のランダムプライマー（ $1\mu\text{g}/\mu\text{l}$ ）（宝酒造社製）、および必要量のDEPCを含む滅菌超純水で $48\mu\text{l}$ の溶液を調製した。この溶液を 65°C で15分間、インキュベートし、反応終了後氷上に置いた。 $24\mu\text{l}$ の5xFirst Strand Buffer（GIBCO社製）、 $12\mu\text{l}$ の0.1M DTT（GIBCO社製）、 $30\mu\text{l}$ のdNTPs（宝酒造社製）、 $4\mu\text{l}$ のSuper Script II reverse tr

anscriptase、および $2\mu\text{l}$ の DEPC を含む滅菌超純水を混合して、 $72\mu\text{l}$ の混合液を調製した。この混合液を前記の氷冷した溶液に加え、総量を $120\mu\text{l}$ とし、 42°C で 1.5 時間、次いで 95°C で 5 分間反応させた。これを -20°C で保存し、PCR 鑄型の母液とした。

【0124】

このように調製した cDNA 溶液を DDW で適当な倍率に希釈し、GAPDH プライマーを用いて、標準化（濃度調整）した。使用した GAPDH プライマーの塩基配列は、下記の通りであった。

5'-ACCTGACCTGCCGTCTAGAA-3' (forward: 配列番号 1077)

5'-TCCACCACCCTGTTGCTGTA-3' (reverse: 配列番号 1078)

【0125】

続いて、DDW で希釈、濃度調整した各サンプルを下記の PCR 反応に供した。

b) PCR 反応

【0126】

PCR 反応は、rTaq polymerase（宝酒造社製）を用いて行った。前記 4 s 期神経芽細胞腫からの cDNA ライブラリーで同定された（新規或いは既知を問わず）遺伝子に対して、適当なプライマーを設計し、濃度調整した 3 組の cDNA サンプル集団のディファレンシャルスクリーニングを行った。すなわち、 $2\mu\text{l}$ の cDNA、 $5\mu\text{l}$ の滅菌蒸留水、 $1\mu\text{l}$ の $10\times$ rTaq バッファー、 $1\mu\text{l}$ の 2mM dNTPs、各々 $0.5\mu\text{l}$ の合成プライマーセット（forward および reverse）、 $0.5\mu\text{l}$ の rTaq を混合した。この混合液を 95°C で 2 分間変性させた後、 95°C 、15 秒間、 58°C 、15 秒間、 72°C 、20 秒間を 1 サイクルとして 35 サイクル繰り返し、さらに 72°C で 20 分間放置し、PCR 反応を行った。使用するプライマーセットによって、バンドが現れなかった場合、サイクル数を増加して、PCR 条件を検討し、それぞれのプライマーのアニーリング温度とサイクル数を決定できた。

【0127】

このように設定した条件で PCR を行った産物を 1.5% アガロースゲルで 2

0 分間電気泳動し、エチジウムブロミドで染色して、3 組の検体（4 s 期神経芽細胞腫、予後良好型の神経芽細胞腫、および予後不良型の神経芽細胞腫）におけるバンドの濃度を比較した。

【0128】

得られた発現パターンを検体サブセット間で、まとめたものが既出の表 1 である。また、発現パターンの解析の結果は、既に議論した通りである。

【0129】

なお、使用したプライマーは、検出しようとする遺伝子の末端シーケンス（実施例 3）をPrimer3ソフトに入力して、適当なプライマー選択条件（塩基数、 T_m 、GC%）で選定した。前出の特定クローンに対応するプライマー配列は、配列表（配列番号175～1076）に与えられている。

【0130】

【発明の効果】

以上説明したように、本発明の遺伝子または本発明の核酸から得られる情報を利用することにより、検定する臨床組織サンプルから該遺伝子を検出して、神経芽細胞腫の予後診断（主に 4 s 期神経芽細胞腫の判定）が可能となる。具体的には、前記遺伝子若しくは核酸から得られる情報を腫瘍マーカーに利用することにより、予後診断に使用可能な、診断剤の調製或いは診断用核酸マイクロアレイを設計することが可能となる。

【0131】

4 s 期神経芽細胞腫の正しい診断ができれば、対象患者に治療が必要かどうかの判断の重要な情報となり、場合によれば不必要な外科手術を避けることができる。

【配列表】

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<120> Nucleic acids isolated from stage 4s neuroblastoma

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<160> 1082

<170> PatentIn Ver. 2.1

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<223> nbla21251

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<213> Homo sapiens

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<223> nbla21480

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1833

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<400> 89

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<211> 1479

<212> DNA

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<220>

<223> nbla21878

<400> 90

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<210> 91

<211> 1907

<212> DNA

<213> Homo sapiens

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<223> nbla21922

<400> 91

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<211> 1402

<212> DNA

<213> Homo sapiens

<220>

<223> nbla22004-2

<400> 92

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<211> 1577

<212> DNA

<213> Homo sapiens

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<400> 93

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<212> DNA

<213> Homo sapiens

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<223> nbla22085

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<211> 1551

<212> DNA

<213> Homo sapiens

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<223> nbla22119

<400> 95

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<211> 2151

<212> DNA

<213> Homo sapiens

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<223> nbla22149

<400> 96

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<213> Homo sapiens

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<223> nbla23956

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<213> Homo sapiens

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23

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24

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22

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<223> Synthetic Primer: nbla23300-1r

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20..

<210> 1075

<211> 21

<212> DNA

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20

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gggactcacc cattttctat tt

22

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20

<210> 1078

<211> 20

<212> DNA

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<220>

<223> Synthetic Primer: GAPD reverse

<400> 1078

tccaccaccc tgttgctgta

20

<210> 1079

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<212> RNA

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<400> 1079

agcaucgagu cggccuuggc cuacugg

27

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<211> 42

<212> DNA

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<220>

<223> Synthetic primer: oligo-dT adapter

<400> 1080

gcggctgaag acggcctatg tggccttttt tttttttttt tt

42

<210> 1081

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer: forward

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agcatcgagt cggccttggt g

21

<210> 1082

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer: reverse

<400> 1082

gcgctgaaga cggcctatgt

20

【書類名】 要約書

【要約】

【課題】 4 s 期神経芽細胞腫に特徴的な遺伝子を同定し、それら遺伝子の核酸配列情報に基づき、神経芽細胞腫の予後（特に、進行度分類および 4 s 期神経芽細胞腫の判定）を診断する。

【解決手段】 配列表の配列番号 1 ないし 174 に記載の核酸配列からなる群より選ばれる 1 つの配列からなる核酸若しくはその断片等、或いはその組み合わせを利用した核酸プローブ、プライマーまたは核酸マイクロアレイからなる、神経神経芽細胞腫の予後診断剤および診断キットを用いて、神経芽細胞腫の予後（特に、進行度分類および 4 s 期神経芽細胞腫の判定）を診断する。

【選択図】 なし

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